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Possible Transmission of Parvovirus B19 From Intravenous Immune Globulin

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To look for genetic changes in human parvovirus B19 that might be associated with chronic infection, we sequenced B19 DNA obtained from serum specimens collected over an approximately 1-year period from a patient with systemic vasculitis. A comparison of the nucleotide sequences of the VP1/VP2 gene from four specimens revealed an abrupt change in the B19 genotype that coincided with initiation of intravenous immune globulin (IVIG) therapy. We suspect that one or more of the lots of IVIG administered to the patient were contaminated with B19. If true, this finding suggests that investigators must be careful in linking B19 infection to disease based on detection of B19 DNA in persons who have received multiple unit blood products. ***J. Med. Virol. 53:233–236, 1997.***

KEY WORDS: PCR; blood products; chronic B19 infection

Human parvovirus B19 has been associated with a wide spectrum of clinical diseases [Török, 1997]. Although infection with this virus usually resolves spontaneously, chronic B19 infections have been reported in persons with immunodeficiencies, and rarely in persons with apparently normal immunological functions [Frickhofen and Young, 1989]. Efforts to attribute chronicity and diversity of clinical manifestations to genetic variants of B19 have been unsuccessful, although greater sequence variability has been reported in persons with persistent B19 infection. Umene and Nunoue [1992] and Hemauer et al. [1996] reported greater relative sequence variability over limited regions of the B19 structural (VP1/VP2) and nonstructural (NS1) protein genes obtained from persons with persistent B19 infections as compared with persons with acute infections. Also, Kerr and colleagues [1995] identified nucleotide (nt) differences in selected regions of the NS1 gene among sequential isolates from persons with persistent B19 infections. Alternatively, Gallinella et al. [1995] found no variation in a 1000-nt region of the VP1/VP2 gene among multiple sequences obtained over a 16-month period from a person with chronic anemia. Similarly, we found that sequences of the complete VP1/VP2 gene obtained at onset of illness (transient aplastic crisis or erythema infectiosum) and approximately 1 month later were essentially identical [Erdman et al., 1996].

As part of our studies of the genetic variability of B19, we sequenced the VP1/VP2 gene from B19 DNA extracted from four serum specimens collected at varying intervals over a period of approximately 1 year from a child with systemic necrotizing vasculitis and apparent chronic B19 infection [Finkel et al., 1994]. Briefly, a 5-year-old boy presented in November 1988, with a 4-month history of unexplained fever, rash, arthralgia, weight loss, and progressive muscle weakness. Skin and muscle biopsies revealed multi-focal necrotizing vasculitis consistent with a diagnosis of polyarteritis nodosa. Immunosuppressive therapies were initiated, but the child remained symptomatic. In August 1989, serum samples were collected and tested for evidence of parvovirus B19 infection. B19-specific immunoglobulin (Ig) G and IgM antibodies were detected by enzyme immunoassay and B19 DNA was amplified by polymerase chain reaction (PCR) [Durigon et al., 1993]. Muscle tissue obtained earlier in the course of illness was also tested by PCR and found to be positive for B19 DNA. B19 DNA remained detectable in serum and bone marrow samples collected between September 1989 and February 1990 (Table I). B19-specific IgM and IgG antibodies declined to undetectable levels by the end of September 1989 and August

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TABLE I. Detection of B19 Specific Antibodies and DNA in Serial Clinical Specimens

ID no.	Specimen	IVIG lot[b]	Date collected[c]	Days post onset	B19 antibody		B19 DNA[d]	
					IgG	IgM	PCR	nPCR
5812	skin		10/07/88	~120			−	−
8056	muscle		11/03/88	147			−	+
—	serum		08/xx/89		+	+	+	+
5246[a]	serum		09/01/89	362	+	−	+	+
5245	serum		09/27/89	388	+	+	−	+
5787[a]	serum		02/05/90	519	+	−	+	+
5789	bone marrow		02/05/90	519			+	+
5927	serum	A >	02/25/90	539	+	−	−	−
5928	serum	B >	02/26/90	540	+	−	−	−
6410	serum	C >	03/25/90	567	+	−	−	−
6804	serum	D, E >	04/28/90	601	+	−	+	+
6805[a]	serum		04/30/90	603	+	−	+	+
7004	serum		06/07/90	641	+	−	+	+
7795[a]	serum		08/23/90	718	−	−	+	+
8712	serum		04/18/91	956	−	−	−	−
8985	serum		06/15/91	1014	−	−	−	−
9861	serum		08/13/92	1439	−	−	−	−
9862	serum		09/24/92	1481	−	−	−	−
10621	serum		01/20/94	1964	−	−	−	−
11128	serum		04/28/94	2062	−	−	−	−
11313	serum		09/14/94	2201	−	−	−	−
11315	serum		09/19/94	2206	−	−	−	−
2411	serum		10/31/94	2248	+	−	−	−

[a]B19 DNA sequenced from indicated serum specimens.
[b]IVIG lots A, B, and C were administered on the corresponding specimen collection date. Lots D and E were administered one day following the specimen collection date.
[c]The patient was symptomatic throughout the shaded period.
[d]B19 DNA was amplified by the polymerase chain reaction (PCR) and confirmed by nested PCR (nPCR) assays.

1990, respectively. Intravenous immune globulin (IVIG) therapy was initiated on February 25, 1990, and consisted of the administration of five separate lots of IVIG solution (1 g/kg Sanglobulin) over a 2-month period. By April, 1990, the child's signs and symptoms resolved. B19 DNA was not detected in a serum sample collected immediately prior to administration of the first lot of IVIG or in two serum samples collected before and after administration of the second lot of IVIG. However, four subsequent serum samples collected after administration of the third, fourth and fifth lots of IVIG were positive for B19 DNA.

B19 DNA was PCR amplified from four serum specimens collected approximately 12, 17, 20 and 24 months after onset of symptoms (Table I). The PCR amplified DNA was then directly sequenced in both forward and reverse directions from two separate DNA extractions of the original samples. Oligonucleotide primer sequences and PCR and sequencing protocols were as previously described [Erdman et al., 1996]. Nucleotide sequences were identical for all extract pairs and there was no evidence of nucleotide heterozygosity at positions where nucleotide substitutions were observed.

As shown in Table II, the VP1/VP2 gene sequences of the first and second isolates were identical and differed from the consensus sequence by 12-nt substitutions. In contrast, sequences 3 and 4 differed significantly from the preceding sequences (14- and 16-nt differences, respectively) and from each other (14-nt differences). These differences were more consistent with the degree of variation we have previously seen among multiple B19 strains obtained from geographically and temporally distinct settings [Erdman et al., 1996].

Efforts to PCR amplify B19 DNA from small aliquots available from each of the IVIG lots had limited success. B19 DNA was amplified from one lot of IVIG (B) by nested PCR using primers to the B19 NS1 gene. However, repeated attempts to amplify DNA from various regions of VP1/VP2 using different primer pairs was unsuccessful. Additional samples of IVIG from these lots were unavailable.

Explanations for the abrupt change in B19 sequences between the first and last two samples from this patient include, i) laboratory contamination of the serum specimens, ii) genetic evolution or immune selection of B19 variants, iii) reinfection by natural exposure and iv) reinfection from B19 contaminated IVIG. We feel that specimen contamination in our laboratory is unlikely; procedures in place minimize the chance of specimen contamination, negative control specimens included in all tests were negative, and the three unique sequences identified (1/2, 3 and 4) were different from other B19 strains we have sequenced in our laboratory. These data do not support a model of genetic evolution of the virus; the rate of nucleotide substitutions was greater than would be expected for a DNA virus like B19; the rate of substitutions was discontinuous, with no changes detected in the first 5 months, followed by abrupt changes over the next 2 to 6 months; and the substitutions were not cumulative, as would be expected if the sequences were derived

TABLE II. Nucleotide Differences in the B19 VP1/VP2 Gene

			Sequence no. and collection date			
nt no.[a]	Consensus nt sequence[b]	aa[c]	1 09/01/89	2 02/05/90	3 04/30/90	4 08/23/90
2453	G/a	E>K	A	A	—	—
2500	G/c	syn	—	—	A	—
2658	A	N>T	C	C	—	—
2701	G	syn	A	A	—	—
2762	A/g	N>D	G	G	—	—
3232	A/g	syn	G	G	—	—
3313	C	syn	T	T	—	—
3391	T	syn	—	—	C	—
3415	A	syn	—	—	G	—
3541	A	syn	G	G	G	—
3544	A/g	syn	G	G	—	—
3877	A/g	syn	G	G	—	—
3997	G	syn	—	—	C	—
4078	A	syn	—	—	G	—
4111	A	syn	—	—	T	—
4132	A/g	syn	G	G	G	—
4312	A	syn	—	—	—	G
4316	A	T>S	—	—	—	T
4498	A/g	syn	—	—	—	G
4570	T/c	syn	C	C	C	—
4591	T/c	syn	C	C	C	—
4720	A/g	syn	—	—	—	G
Total nt and aa differences from the consensus sequence (nt/aa):			12/3	12/3	10/0	4/1

[a]Nucleotide (nt) numbering based on Shade et al., (1986).
[b]Consensus nt sequence based on previous report (Erdman et al., 1996). Upper case bases represent the predominant bases identified among study sequences at the indicated positions; lower case bases represent the minority bases at the same positions.
[c]Synonymous (syn) and deducted amino acid (aa) substitutions.

from a common lineage. The abrupt change in the sequences could possibly be explained by the introduction of selective immune pressure following administration of the IVIG. However, this seems unlikely, as only 4 of the nucleotide changes identified among the post-IVIG strains resulted in predicted amino acid changes that could potentially affect the antigenic composition of the virus, and 3 of these changes (nt positions 2458, 2658, 2762) were to amino acids commonly found among B19 strains [Erdman et al., 1996]. It is, therefore, likely that the post-IVIG strains would also have been neutralized by the IVIG used to treat this patient. Although reinfection could have occurred from community exposure, it seems unlikely that the patient would be repeatedly infected with B19 (to account for two distinct sequences) after the initial infection. Since IVIG has been shown to frequently contain B19 DNA [Saldanha and Minor, 1996], and given the coincidental appearance of the new B19 strains after administration of the IVIG, we feel that the IVIG was the most likely source of infection. Our failure to amplify sufficient B19 DNA from the IVIG for sequencing may reflect the presence of low titer virus and our inability to adequately sample the IVIG retention samples. Nevertheless, our ability to detect B19 DNA by nested PCR from one lot of IVIG is consistent with, but does not confirm, our suspicion that the IVIG was the source of the B19.

B19 is not commonly found in individual blood donations, 1 in 1000 to 50,000 units, depending on the detection system [Mortimer, 1983; Cohen et al., 1990; McOmish et al., 1993; Jordan et al., 1996]. However, because virus levels in blood can exceed 10^{11} particles/ml, and plasma pools used to produce plasma derivatives (albumin, factor VIII and XI, and IVIG) often contain >5000 blood donations, the potential for B19 contamination of these blood products is high. In a recent study, B19 DNA was found in 60% to 100% of plasma start pools from individual manufacturers in the United Kingdom and 20% of treated IVIG samples [Saldanha and Minor, 1996]. Its small size and ability to withstand heat and detergent treatments has thus far impeded efforts to remove or inactivate B19 during manufacture of blood products [Lyon et al., 1989; Morfini et al., 1992; Große-Bley et al., 1994].

Ironically, antiviral therapy with IVIG has been recommended for the treatment of chronic B19 infection in immunosuppressed patients with associated anemia [Anderson, 1997]. In most instances, B19 antibodies present in IVIG would probably neutralize both the patient's virus and any virus in the IVIG preparation, resolving the ongoing infection and preventing clinically apparent reinfection. It is possible, however, that the virus and specific antibodies form immune complexes that enabled infection of macrophages, monocytes, or other cells with Fc receptors, as has been shown to occur in vitro by Morey et al. [1992]. These cells, at most, will only support very limited replication of the virus and probably a short-term limited infection that may be clinically unapparent. Although the patient described in this study did not appear to have any new B19-associated illness while viremic with strains 3

and 4, further study is needed to clarify the clinical implications of reinfection in the presence of antibody. Even though these infections may not be clinically relevant, they apparently can produce a low titer viremia that can confound attempts to document chronic infection and associations between B19 and disease. In light of these findings, it is important that positive B19 PCR results obtained from persons receiving blood products be interpreted with care.

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